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Are astrocytes the pressure-reservoirs of lactate in the brain?

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Abstract

The role of lactate in the brain has been controversial. In this issue, Machler et al (2015) present data from *in vivo* experiments suggesting that there is a downwards gradient of lactate in the brain. They provide a mechanistic basis for the proposed export of lactate from astrocytes to neurons.

Main body

The hypothesis of "lactate shuttle" is approximately 20 years old but is still surrounded by controversies (Dienel, 2012). In a nutshell, it poses that in addition to direct consumption of glucose, neurons consume significant amount of lactate, which is produced by astrocytes, numerous non-excitable cells of the brain. Neurons are extremely efficient in uptake and catabolism of glucose(Lundgaard *et al.*, 2015) and it is not immediately clear why consumption of lactate should be beneficial for them if glucose levels are normal. Possibly, bypassing glycolysis gives neurons some advantage during bouts of activity when ATP

consumption suddenly increases and mitochondria urgently need pyruvate. Another explanation is that uptake of lactate and conversion into pyruvate could allow neurons to replenish their stores of NADH, as transformation of lactate into pyruvate leads to conversion of NAD+ into NADH. Whatever the reason, it seems that interfering with the flux of lactate from astrocytes into neurons affects brain function, for example it interferes with memory formation in chick and rodents(Gibbs *et al.*, 2006;Suzuki *et al.*, 2011).

Irrespective of the actual benefits of lactate shuttling from astrocytes to neurons, a critical pre-requisite for this process is the existence of a downwards gradient, because all known mechanisms of lactate movement are passive. Initially it was thought that such gradient appears when astrocytes upregulate lactate production in response to a chemical signal from activate neurons, but the study by Machler and colleagues (Mächler *et al.*, 2015) demonstrates that a gradient exists even in the absence of stimulation. The main tool that was used for estimating lactate concentrations was a genetically encoded FRET lactate sensor laconic where cyan and yellow proteins are separated by a flexible, metabolite-sensitive linker. Laconic provides cellular resolution and the authors made a bold step to perform their experiments *in vivo* using anesthetized mice. Laconic was targeted to either neurons or astrocytes using viruses with cell-specific promoters, which were injected into the adjacent parts of the cortex. By moving the field of view, it was possible to make observations on either class of cells in the same animal. There are not many studies where anything like this has been done and this approach is definitely a big technical step forward.

The authors build their arguments based on the results of two types of experiments. First, they attempt to saturate laconic using lactate load and then compare the magnitude of change between the two cell types. In some experiments, lactate infusions are combined

with NH4Cl, with the aim to suppress pyruvate consumption by acting on mitochondria (Felipo & Butterworth, 2002;Lerchundi *et al.*, 2015). Alternatively, they try to wash lactate out using a "trans-acceleration" approach. For trans acceleration, they create an excess of extracellular pyruvate, which should lead to a strong inward flux though the monocarboxylate transporters (MCTs), thus accelerating pick up of lactate from the inside of the cell. The trans-acceleration method is not without caveats. First of all, it is hard to see why a sudden rise in intracellular pyruvate should not result in its quick transformation into lactate, which, given the differences in lactate dehydrogenase (LDH) isoforms between astrocytes and neurons (Mosienko *et al.*, 2015), may happen at different rates. Second, as was first seen in (San *et al.*, 2013), pyruvate seems to directly antagonize lactate interaction with laconic. Nevertheless, the paper shows that pyruvate load leads to a reduction of laconic signal in astrocytes, but not in neurons. In fact, after a delay, neuronal laconic signal went up, suggesting that lactate which had been exported from astrocytes, then reappeared in neurons.

Using laconic for quantitative comparisons between two cellular populations is not trivial. Fluorescent proteins used in laconic become visible when their concentration in the cell reaches high nanomolar range (Stokes *et al.*, 2003). Lactate concentrations even in resting cells are much higher. This is quite different from the typical Ca^{2+} imaging experiments where the indicator is often in excess. In other words, the limit of the laconic response is not set by the availability of free indicator but by the stoichiometry of lactate-laconic interaction, which seems to be exponential. Since all measurements were made in non-stationary conditions, factors such as speed and affinity of MCTs, differences in LDH isoforms, rate of pyruvate consumption which are all different between neurons and astrocytes, need to be taken into account when considering the results of experiments with

lactate loading and trans-acceleration. The authors have provided various controls to support their interpretation and the readers should look carefully at the arguments in the paper and the supplement.

Non-equilibrium of lactate concentration at rest (Fig 1B) implies that in spite of the presence of MCTs on the astrocytic and neuronal membranes, the transporters are unable to evenly distribute lactate between these cellular compartments. Possibly this is a consequence of a low affinity of astrocytic MCTs for lactate which to some extent acts like a threshold and only when lactate production rises in astrocytes, it "spills" over. If this is this case, the alternative mechanisms of "gated" lactate exit such as exit via hemichannels may become important.

It is interesting to note that for laconic to work, *in vitro* cultures have to be kept in solutions with 1 mM glucose (the usual concentration used for slice and culture work is 10 mM). Does that mean that the absolute majority of papers on brain slices are made under conditions when both, astrocytes and neurons are overloaded with lactate?

We still do not have a convincing explanation why neurons should benefit from importing lactate. Now that we are getting these new results using *in vivo* imaging with cellular resolution, the ultimate purpose of lactate import from astrocytes into neurons becomes a highly pertinent question.

Reference List

Dienel GA (2012). Brain lactate metabolism: the discoveries and the controversies. *J Cereb Blood Flow Metab* **32**, 1107-1138.

Felipo V & Butterworth RF (2002). Mitochondrial dysfunction in acute hyperammonemia. *Neurochem Int* **40**, 487-491.

Gibbs ME, Anderson DG, & Hertz L (2006). Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. *Glia* **54**, 214-222.

Lerchundi R, Fernandez-Moncada I, Contreras-Baeza Y, Sotelo-Hitschfeld T, Machler P, Wyss MT, Stobart J, Baeza-Lehnert F, Alegria K, Weber B, & Barros LF (2015). NH4+ triggers the release of astrocytic lactate via mitochondrial pyruvate shunting. *Proc Natl Acad Sci U S A* **112**, 11090-11095.

Lundgaard I, Li B, Xie L, Kang H, Sanggaard S, Haswell JD, Sun W, Goldman S, Blekot S, Nielsen M, Takano T, Deane R, & Nedergaard M (2015). Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat Commun* **6**, 6807.

Mächler P, Wyss MT, Elsayed M, Stobart J, Gutierrez R, Zuend M, San MA, Romero-Gomez I, Baeza-Lehnert F, Lengacher S, Schneider BL, Aebischer P, Magistretti PJ, Barros LF, & Weber B (2015). In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell Metab*.

Mosienko V, Teschemacher AG, & Kasparov S (2015). Is L-lactate a novel signaling molecule in the brain? *J Cereb Blood Flow Metab* **35**, 1069-1075.

San MA, Ceballo S, Ruminot I, Lerchundi R, Frommer WB, & Barros LF (2013). A Genetically Encoded FRET Lactate Sensor and Its Use To Detect the Warburg Effect in Single Cancer Cells. *PLoS ONE* **8**, e57712.

Stokes CEL, Murphy D, Paton JFR, & Kasparov S (2003). Dynamics of a transgene expression in acute rat brain slices transfected with adenoviral vectors. *Experimental Physiology* **88**, 459-466.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, & Alberini CM (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* **144**, 810-823.